Guidance for Industry

In Vivo Pharmacokinetics and Bioavailability Studies and In Vitro Dissolution Testing for Levothyroxine Sodium Tablets

Draft Guidance

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U.S. Department of Health and Human Services
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Center for Drug Evaluation and Research (CDER)
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GUIDANCE FOR INDUSTRY[1]

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I. INTRODUCTION

This guidance is intended to assist sponsors of new drug applications (NDAs) for levothyroxine sodium tablets who wish to conduct in vivo pharmacokinetic and bioavailability studies and in vitro dissolution testing for their products. Information from these studies would generally be submitted in Section VI of an NDA. Sponsors who wish to use approaches other than those recommended in this guidance should discuss their plans with the FDA prior to preparing an NDA.

II. BACKGROUND

Levothyroxine sodium is the sodium salt of the levo isomer of the thyroid hormone, thyroxine. Thyroid hormones affect protein, lipid, and carbohydrate metabolism; growth; and development. They stimulate the oxygen consumption of most cells of the body, resulting in increased energy expenditure and heat production, and possess a cardiostimulatory effect that may be the result of a direct action on the heart.

The production of levothyroxine hormone is regulated by the hypothalamus-pituitary axis through a negative feedback system. When hormone levels are inadequate, the hypothalamus secretes thyroid stimulating hormone-releasing hormone (TSH-RH), which stimulates the anterior pituitary to produce thyroid stimulating-hormone (TSH). TSH then stimulates the thyroid gland to produce levothyroxine (T_4) and triiodothyronine (T_3). T_4 is subsequently converted to the highly

[1] This guidance has been prepared by the Division of Pharmaceutical Evaluation II, Office of Clinical Pharmacology and Biopharmaceutics, which operates under the direction of the Office of Pharmaceutical Science in the Center for Drug Evaluation and Research (CDER) at the Food and Drug Administration (FDA). The guidance has also been reviewed by the Guidances Technical Committee of the Biopharmaceutics Coordinating Committee, as well as the Division of Metabolic and Endocrine Drug Products in CDER. This guidance document represents the Agency's current thinking on the pharmacokinetics/bioavailability/dissolution information that should be submitted in a new drug application (NDA) on levothyroxine sodium tablets. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. An alternative approach may be used if such approach satisfies the requirements of the applicable statute, regulations, or both.

active T_3 in the peripheral tissues. High levels of T_4 inhibit the production of TSH and (to a lesser degree) TSH-RH. This effect in turn decreases the further production of T_4 (Farwell 1996).

Orally administered levothyroxine sodium is used as replacement therapy in conditions characterized by diminished or absent thyroid function such as cretinism, myxedema, nontoxic goiter, or hypothyroidism. The diminished or absent thyroid function may result from functional deficiency, primary atrophy, partial or complete absence of the thyroid gland, or the effects of surgery, radiation, or antithyroid agents. Levothyroxine sodium may also be used for replacement or supplemental therapy in patients with secondary (pituitary) or tertiary (hypothalamic) hypothyroidism.

Levothyroxine sodium is a compound with a narrow therapeutic index. If a drug product of lesser potency or bioavailability is substituted in the regimen of a patient who has been controlled on another product, a suboptimal response and hypothyroidism could result. Conversely, substitution of a drug product of greater potency or bioavailability could result in toxic manifestations of hyperthyroidism such as cardiac pain, palpitations, or cardiac arrhythmias. In patients with coronary heart disease, even a small increase in the dose of levothyroxine sodium may be hazardous. Hyperthyroidism is a known risk factor for osteoporosis (Paul et al. 1988). To minimize the risk of osteoporosis, it is advisable that levothyroxine sodium be titrated to the lowest effective dose. Because of the risks associated with over- or undertreatment with levothyroxine sodium, it is critical that patients have available to them products that are consistent in potency and bioavailability.

It is a challenge to determine the bioavailability of levothyroxine sodium products because levothyroxine is naturally present in minute quantities in the blood, with the total levels reaching 5.0-12.0 μg/dl and free (or unbound) levels reaching 0.8-2.7 ng/dl in a healthy adult. To assess the bioavailability of levothyroxine sodium after a single dose, several times the normal dose should be given to raise the levels of the drug significantly above baseline to allow measurement. Furthermore, levothyroxine has a long half-life of 6 to 9 days, and therefore, a long washout period is necessary between treatments.

III. PHARMACOKINETICS AND BIOAVAILABILITY STUDIES IN VIVO

Information on the pharmacokinetics (absorption, distribution, metabolism, and excretion) of levothyroxine sodium can be obtained from the literature and/or from original studies. If the studies cited have used levothyroxine sodium formulations other than the formulation intended for market, the submission should contain information identifying how those formulations differ from the to-be-marketed formulation.

For sponsors who have a product on the market, we recommend that in vivo bioavailability studies be conducted using the formulation(s) already on the market, assuming that a sponsor intends to keep marketing the formulation(s). The tablets used in the study should be made from a full-scale production batch and should meet all compendial requirements. The formulations used should demonstrate sufficient stability for the length of the study. Stability evaluations should be made for the bio-batch prior to and after the study. All dissolution, potency, and content uniformity data should be submitted to the NDA for review.

For sponsors who do not have a levothyroxine sodium formulation on the market, the usual approaches to developing pilot-scale batches for bioavailability studies apply. [2]

A. Inclusion Criteria

For each pharmacokinetics and bioavailability study outlined below, at least 24 volunteers should complete the trial. The subjects should be healthy volunteers, 18 to 50 years of age and within 15 percent of ideal body weight for their height and build. Sponsors should attempt to enroll approximately equal numbers of men and women. Volunteers recruited for the study should have an acceptable medical history, physical examination, and clinical laboratory tests. All thyroid function tests should be within normal limits. Volunteers with any current or past medical condition that might significantly affect their pharmacokinetic or pharmacodynamic response to levothyroxine sodium should be excluded. Female volunteers should be given a pregnancy test prior to beginning the study. Pregnant women should be excluded from the study. Written informed consent must be obtained from all volunteers before they are accepted into the study.

B. Single-Dose Bioavailability Study

Objective: To determine the bioavailability of the to-be-marketed formulation of levothyroxine relative to a reference (oral solution) under fasting conditions.

Design: The study is a single-dose, two-treatment, two-sequence crossover design. An equal number of volunteers should be randomly assigned to each sequence. The washout period between treatments should be at least 35 days.

Dose and Number of Studies: One bioavailability study using multiples of the highest tablet strength to achieve a total dose of 600 µg will be sufficient provided that:

1. The tablet strengths differ only in the amount of levothyroxine sodium and filler needed to maintain the tablet weight,

[2] See *Q1A Stability Testing of New Drug Substances and Products (59 FR 48754, September 1994).*

2. Multi-point dissolution profiles are similar across tablet strengths, and

3. The results of the dosage-form equivalence study (see Section C) indicate that the tablets studied are equivalent.

Sponsors whose products do not meet these should contact the Division of Pharmaceutical Evaluation II for further guidance.

Procedure: Following a 10-hour overnight fast, volunteers should be administered a single dose of levothyroxine sodium orally with 240 mL water. The treatments should be as follows:

Treatment 1: Multiples of the highest levothyroxine sodium tablet to be marketed .

Treatment 2: Levothyroxine sodium as an oral solution at an equivalent dose with treatment 1. The intravenous formulation can be used as a convenient source of an oral levothyroxine solution.

Volunteers should remain fasted for 4 hours after dosing, with water only allowed after the first hour. Volunteers should be served standardized meals according to the schedule throughout the study.

Blood Sampling: Blood samples should be drawn at the following times after oral dosing: -0.5, -0.25, 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 18, 24, and 48 hours post dose.

Data Analysis: Individual and mean plasma/serum concentration-time profiles of total (bound + free) T_4 and T_3 should be included in the report. The following pharmacokinetic parameters should be computed:

- Area under the plasma/serum concentration-time curve from time 0 to the last measurable time point (AUC_{0-t})
- Peak concentration (Cmax)
- Time to peak concentration (Tmax)

Analysis of variance (ANOVA) should be performed for log-transformed AUC_{0-t} and Cmax data using the SAS General Linear Models (GLM) procedure. The oral solution should be used as the reference formulation. The geometric means and 90 percent confidence intervals of the AUC_{0-t} and Cmax ratio (test/reference) should be presented as evidence of bioavailability.

C. Dosage-Form Equivalence Study

Objective: To determine the dosage-form equivalence among the to-be-marketed tablet strengths of levothyroxine sodium.[3]

Design: The recommended study is a single-dose, three-treatment, six-sequence crossover design. An equal number of volunteers should be randomly assigned to each sequence. The washout period between treatments should be at least 35 days.

Tablet Strengths and Dose: Three strengths of tablets should be studied that represent the low, middle, and high strength of the formulations to be marketed. Generally, the middle strength studied is the 100 µg tablet. Multiples of each tablet strength are necessary for detection of T_4 above baseline levels. The total dose given for each treatment in the study will usually be 600 µg and should be the same dose for each treament.

Procedure: Following a 10-hour overnight fast, volunteers should be administered a single dose of levothyroxine sodium orally with 240 mL water. The treatments are as follows:

Treatment 1: Multiples of the representative low strength tablet (usually 50 µg).

Treatment 2: Multiples of the representative mid-strength tablet. This is normally the 100 µg tablet, and should be considered as the reference for this study.

Treatment 3: Multiples of the representative high strength tablet (usually 300 µg).

Volunteers should remain fasted for 4 hours after dosing, with water only allowed after the first hour. Volunteers should be served standardized meals according to the schedule throughout the study.

Blood Sampling: The blood sampling schedule for this study should be identical to that recommended for the bioavailability study.

Data Analysis: Individual and mean plasma/serum concentration-time profiles of total (bound + free) T_4 and T_3 should be included in the report.

The pharmacokinetic parameters, including AUC_{0-t}, Cmax and Tmax, should be computed for both total T_4 and T_3. For the assessment of equivalence between dosage forms, the log-transformed AUC_{0-t} and Cmax data should be analyzed with ANOVA using the SAS GLM procedure. The geometric means and 90% confidence intervals of the ratio of AUC_{0-t} and Cmax

[3] Available strengths of levothyroxine sodium tablets from many manufacturers include 25, 50, 75, 88, 100, 112, 125, 137, 150, 200 and 300 µg.

should be presented for each pairwise comparison. Dosage-form equivalence is demonstrated if the 90% confidence intervals fall within the 80-125% range.

For both single-dose bioavailability and dosage-form equivalence studies, the assessment of bioavailability should be based on the measurement of total (bound + free) T_4 and total T_3 levels. The determination of free T_4 and T_3 is not necessary. However, if sufficiently precise and accurate assays are available for free T_4 and T_3, these moieties can be measured as well. Statistical analyses of free T_4 and T_3 should then be performed, with the results used as supportive data. If free T_4 and T_3 are measured, the assays used should be based on the immuno-extraction (two-step) method, rather than the labeled analog (one-step) method. Levels of thyroid-stimulating hormone (TSH) should be measured as part of the volunteer screening process as well as post-study. These TSH data should be reported in the NDA.

IV. DISSOLUTION TESTING IN VITRO

Dissolution studies can be performed using the current USP method or others provided that justification for the choice of the method(s) is given. For each tablet strength to be marketed, multi-point dissolution studies should be performed on three production-sized batches using 12 tablets per batch. The time points that should be used are 10, 20, 30, 45, 60, 80, 100, and 120 minutes, or until 80 percent of the labeled claim is dissolved, so that a complete profile may be obtained. Dissolution testing should include lots used in the bioavailability studies.

V. ASSAY VALIDATION

Assays used for both in vivo and in vitro studies should be reproducible, precise, accurate, specific, stable, and linear. If commercial kits are used, they should be validated in-house at the analytical site where the assay for the study is performed. Please note that the validation data from the kit manufacturer alone is insufficient.

VI. FORMULATION

The composition of the formulation for each tablet strength of levothyroxine sodium to be marketed should be provided in the NDA submission.

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